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(54) Title: METHOD FOR QUANTITATIVE DETERMINATION OF FAT IN AN EMULSION WHICH CONTAINS FAT PARTICLES
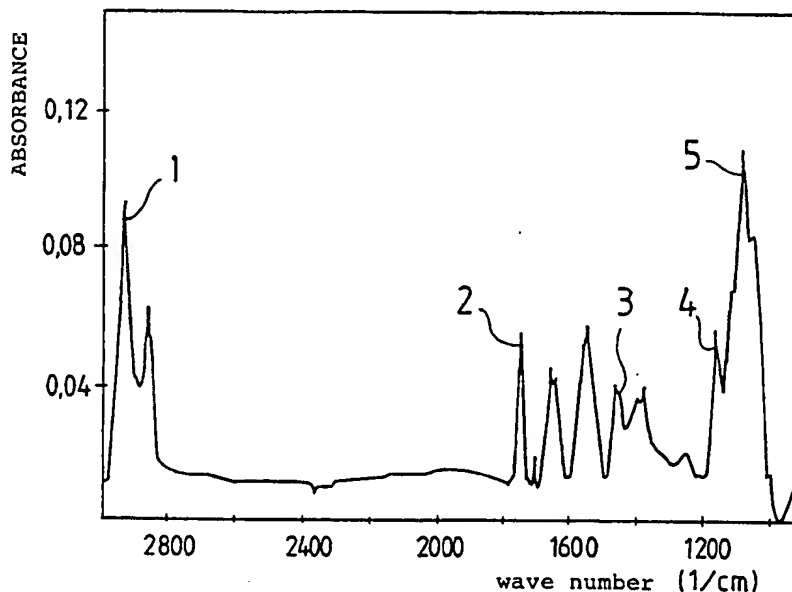
(57) Abstract

The invention relates to a method for quantitative determination of fat from an emulsion, such as milk or a milk product, containing fat particles. The method uses an IR absorption technique in which the determination is based on a specific absorption peak in the absorption spectrum of the emulsion sample, and the essential idea of the invention is that the absorption peak, at a wave number of approx. 1160-1190 (1/cm), of the single carbon-oxygen bonds of the ester groups of the fat molecules is used in the determination. When the determinations are carried out on milk or on a milk product, the effect of lactose can, when so desired, be taken into account by measuring the characteristic absorption peak of lactose and by carrying out, on the basis of it, a correction in the fat concentration value yielded by the C-O absorption peak. Since scatter does not affect the absorption peak of the C-O bond, the determinations according to the invention can be carried out directly from full milk, without a preceding homogenization treatment.

Method for quantitative determination of fat in an emulsion
which contains fat particles

The present invention relates to a method for quantitative
determination of fat in an emulsion which contains fat par-
ticles, in which method the infrared absorption of the emulsion
sample is measured and the fat is determined on the basis of a
specific absorption peak in the absorption spectrum by elimi-
nating from it any error effect possibly caused by the other
constituents of the emulsion.

The principal targets of quantitative fat determinations are
milk and products made from milk. The natural fat concentration
in milk is usually within the range 3.0-5.5 %, but the fat
concentrations in different milk products may vary from close
to 0 to more than 40 %. Measurement of the fat concentration is
required as a basis for the milk price to be paid to the pro-
ducer and additionally in the quality control of products pre-
pared from milk.

The determination of the fat concentration in milk is possible
by traditional chemical means, of which so-called Gerber and
Röse-Gottlieb methods can be mentioned as examples. However,
infrared absorption is currently used in routine determina-
tions, although chemical methods continue to be necessary for
the calibration of the IR analyzers. Most commonly the absorp-
tion peak, at a wave number of approx. 1750 (1/cm) (at a wave-
length of approx. 5.7 μm), of the carbon-oxygen double bonds of
the triglyceride part contained in the fat molecule is used in
IR determinations. In addition to this there is used a method
described in EP Patent publication 0012492, which is based on
the absorption peak, at a wave number of approx. 2870 (1/cm)
(at a wavelength of approx. 3.5 μm), of the carbon-hydrogen
bonds in the fat molecule.

The last-mentioned method, based on the absorption of the C-H bond, has been justified by the fact that the method, contrary to the method based on the C=O double bond, is not dependent on variations in the fat composition of milk, which is due to the fact that the amount of the absorbing C-H bonds in the fat molecule is directly proportional to the size of the molecule. On the other hand, when the C-H bond is used, it is necessary to take into account the contribution to the absorption peak by other substances present in milk, mainly proteins and lactose, which contain C-H bonds. This can be done by measuring, in addition to the absorption peak of the C-H bond also the IR absorption peaks characteristic of the said other substances and by calculating on the basis of them a correction of the C-H bond absorption peak, thereby arriving at the fat concentration aimed at in the determination.

The determination of the fat concentration in milk on the basis of the absorption of the C-H bond does, however, involve problems which are more difficult than the above-mentioned computational correction of the error caused by proteins and lactoses. The first problem to be mentioned is that the error-causing milk constituents which contain C-H bonds are by no means limited to proteins and lactose; dozens of them are present in milk, at concentrations which vary with great random. Some of such constituents are vitamins, nucleic acids, esters of organic acids, lactones, amino acids, amines, and cholesterol. The concentrations of these substances are dependent, among other things, on the breed and state of health of the cow, on its feed, which varies from one season to another, and on the condition of the sample at the time of the analysis, which is dependent on the storage of the sample. The concentration of each individual substance in milk remains usually so low that taking it into account in the determination of fat in the same manner as proteins and lactose is not possible in practice, but the combined total concentration of these substances in milk may rise up to 0.1 %, and in poorly stored samples even higher

than this, in which case they will cause an error of a cor-
responding order of magnitude in the fat concentration calcu-
lated from the C-H absorption peak.

Another problem associated with the use of the C-H absorption
peak is that the peak is located in the infrared spectrum in-
conveniently on the slope of a strong absorption peak of water,
from which it is difficult to distinguish. Furthermore, the
proportion of water in the sample on which the determination is
being performed is not always known precisely, which causes in
the results an uncontrolled error which is further multiplied
in the corrections calculated for proteins and lactose.

A further error source which hampers the use of the C-H bond is
the scatter occurring in the sample alongside IR absorption.
The scatter caused by fat particles is inversely proportional
to the fourth power of the wavelength used, and is additionally
dependent on the ratio of the wavelength to the diameter of the
fat particles in such a manner that, when the wavelength in-
creases to a value higher than the particle diameter, the scat-
ter rapidly decreases to an insignificant level. Scatter thus
constitutes a problem in milk fat determination when the wave-
length of the absorption peak used corresponds to or is smaller
than the diameter of the particles. When the diameter of the
fat particles in raw milk is in the order of 0.5-5 µm, it fol-
lows from this that scatter strongly disturbs C-H bond absorp-
tion occurring at a wavelength of 3.5 µm, unless the fat par-
ticles can be reduced to a diameter clearly less than 3.5 µm,
preferably to a diameter less than 2 µm, in a homogenization
before the fat determination. Therefore it is stated in the
above-mentioned EP patent publication 0012492 that the mean
diameter of the fat particles may be at maximum 2 µm, and
usually the IR analyzers used for milk fat determinations are
indeed equipped with homogenizers by means of which the fat
particles can be chopped to a size range of 0.5-1.5 µm. How-
ever, in continuous use the homogenizer is subject to gradual

wear, whereby the dimensions of its mechanical parts are changed and its operating capacity deteriorated to the point that, with time, more and more poorly homogenized milk samples, containing also fat particles larger than 2 μm in diameter and giving erroneous results, arrive in the measuring cuvette. The problem could be in part diminished by, for example, homogenizing the sample several times, but this would lower the analyzer capacity to such an extent that the system is not practicable.

When milk fat determination is based on the absorption peak of the C=O double bond, the problem of scatter is clearly smaller. This is due to the absorption peak being located at a higher wavelength, 5.7 μm, which exceeds the maximum size of fat particles, which is approx. 5 μm. The absorbance curve depicting the concentration of fat as a function of absorption is clearly more gently sloping with the C=O absorption peak than with the C-H absorption peak, and respectively the precision of the determination is higher, especially at higher concentrations of fat. However, also when the C=O double bond is used, obtaining precise results presupposes the homogenization of the milk before the measuring.

The object of the present invention is to provide a new method for quantitative determination of fat by an infrared absorption technique in which the above-mentioned problem of scatter and also the disadvantages associated with the use of the C-H bond are avoided. The method according to the invention is characterized in that the determination is made from an absorption peak, at a wave number of approx. 1160-1190 (1/cm), of the single carbon-oxygen bonds (C-O) of the ester groups of the fat molecules.

The wave number, approx. 1160-1190, of the IR absorption peak used in accordance with the invention corresponds to a wavelength of approx. 8.4-8.6 μm, which is so much above the maximum size, approx. 5 μm, of fat particles that scatter will no

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longer affect the result of the determination. A comparison of
the previously used C-H and C=O absorption peaks with the C-O
absorption peak used according to the present invention shows
that the effect of scatter on the C-H peak is 34-fold and on
the C=O peak five-fold as compared with the C-O peak. This
means that, in the method according to the invention, the
homogenization of milk before the absorption measurement is
unnecessary. The measurement can be performed from raw milk, in
which mere careful mixing suffices for providing a representa-
tive sample.

From the elimination of the homogenization step there follows
the advantage that the IR analyzer may be simpler in structure
than previously. A homogenizer typically made up of a hole and
a ball spring-actuated against it, and the high-pressure pump
required for it, can be omitted, and a conventional pump oper-
ated at low pressures suffices for the feeding in of the
sample. As the apparatus is simplified, also its maintenance
requirement is reduced, and as the problems due to the wear of
the homogenizer have been eliminated, the results obtained are
more reliable than previously.

The absorption peak, at a wavelength of approx. 1160-1190, of
the C-O bond, used according to the invention, is located in
the IR absorption spectrum at a place considerably more ad-
vantageous with respect to the absorption of water than is the
absorption peak of the C-H bond. When milk or some other water-
based emulsion is measured, the reading of the peak is there-
fore easier and the determination result obtained is not de-
pendent on the amount of water present in the sample.

It is also a significant advantage of the invention that the
C-O absorption peak used is highly specific with respect to
fat. With the exception of certain esters of amino acids or
other organic acids, present incidentally in small amounts,
milk or milk products do not contain any constituents having a

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single C-O bond other than fat. The single C-O bond is superior
in specificity even over the C=O bond, which is present in free
amino acids and other organic acids which are present in milk
in larger amounts than are the above-mentioned esters. This
means that, in the method according to the invention it is not
necessary to use the computational compensations which are
necessary in the determination based on the C-H bond and in
which the contribution of proteins and lactose to the C-H ab-
sorption peak must be taken into account. The only slight cor-
rection requirement in the determination of the fat concentra-
tion in milk by the method according to the invention is due to
a lactose peak which is located close to the C-O absorption
peak and may slightly overlap with the C-O peak, primarily
owing to deficiencies in the resolution of the analyzers. How-
ever, the error in the result will not be great even if the
lactose peak is not taken into account.

In addition to measuring the absorption peak of the C-O bond it
is necessary, regardless of the type of the emulsion, to mea-
sure the absorption at a reference wave number, which may be,
for example, a wave number of approx. 1200 or approx. 1300-
1350. For this reason it may be preferable to measure a con-
tinuous infrared absorption spectrum within a somewhat wider
wave number range, such as a range of approx. 1160-1350.

When determination is performed on milk or on a milk product,
for example, unhomogenized milk which has a fat concentration
of approx. 3.0-5.5 % and in which the fat particle size varies
mainly within the range 0.5-5 μm, it is possible according to
the invention to measure, besides the C-O absorption peak, also
the characteristic absorption peak of lactose at a wave number
of approx. 1050, in which case it is possible, on the basis of
the latter, to eliminate the effect of lactose on the C-O ab-
sorption peak. In this case it is preferable to measure a con-
tinuous IR absorption spectrum within a wave number range of
approx. 1050-1350.

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In the accompanying drawing,

Figure 1 depicts an infrared absorption spectrum of full milk
(fat concentration 3.4 %) measured by means of a Mattsson FTIR
spectrometer within a wave number range of approx. 1000-3000,
and
Figure 2 depicts an infrared absorption spectrum of the tri-
glyceride ester of oleic acid, measured by means of the same
spectrometer within a wave number range of approx. 1000-3000.

In the absorption spectrum according to Figure 1, from which
the effect of the water in the background has been eliminated,
the absorption peak 1 of the C-H bond is seen at a wave number
of approx. 2870, the absorption peak 2 of the C=O double bond
at a wave number of approx. 1750, the absorption peak 3 of the
C-C bond at a wave number of approx. 1440, and the absorption
peak 4 of the single C-O bond at a wave number of approx. 1175.
In addition to the absorption peaks of these bonds belonging to
the fat molecule, the absorption peak of the C-OH bond of lac-
tose at a wave number of approx. 1050 (at a wavelength of ap-
prox. 9.5 μm) is indicated in Figure 1 by reference numeral 5.

In the absorption spectrum of oleic acid triglyceride according
to Figure 2, the same absorption peaks 1-4 due to the different
bonds of fat as in Figure 1 are seen. In the absence of other
absorbing constituents and scatter, the said peaks are in this
case of a more or less equal value as the basis for quantita-
tive determination of fat.

According to the basic idea of the invention, the concentration
of fat in milk is determined from the absorption peak of the
single C-O bond, seen in Figure 1 within a wave number range of
1060-1090, by subtracting from this the absorption at the ref-
erence wave number, which may be, for example, the wave number
1200, and by calculating the fat concentration by using a

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formula having the form

$$F = ax^3 + bx^2 + cx - d$$

where F is the fat concentration and x = absorbance, and possibly by further making, in the result obtained, a correction due to the lactose peak 5. When a DaiLab IR-2000 spectrometer is used, the said calculation formula is

$$F = 35.1x^3 + 28.4x^2 + 26.0x - 0.02$$

and the lactose correction coefficient, by which the absorbance of the peak 5 is multiplied and then added to the fat concentration calculated from the C-O absorbance peak 4, is 0.203.

The significance of homogenization in the quantitative determination of milk fat was tested by measurements using a fixed-lattice DaiLab IR-2000 spectrometer, in which the separate detectors are located at specific points corresponding to the different wavelengths to be measured and in which all the absorbance measurements are performed simultaneously. In the said apparatus it is possible to measure all the absorbances within a short time period 0-140 times in succession and to calculate from their mean the fat concentrations by using predetermined arithmetic formulae.

The following table shows the fat concentrations determined from 10 different milk samples on the basis of the absorption peaks of different bonds in the fat molecule, in each case as the mean of 100 successive individual absorbance measurements. For comparison, each sample was measured both after homogenizer treatment and unhomogenized.

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<u>Table</u>

Bond Wave-length	Sample	Determined fat concentration (%) Homogenized	Determined fat concentration (%) No homogenization
(C-O) 8.5 µm	1	3.42	3.42
	2	3.66	3.66
	3	3.42	3.41
	4	3.42	3.41
	5	4.30	4.29
	6	2.88	2.88
	7	3.72	3.73
	8	4.47	4.47
	9	4.13	4.12
	10	4.10	4.09
(C-C) 7.0 µm	1	3.43	3.43
	2	3.65	3.67
	3	3.43	3.43
	4	3.42	3.42
	5	4.31	4.31
	6	2.89	2.90
	7	3.74	3.76
	8	4.45	4.47
	9	4.11	4.12
	10	4.14	4.14
(C=O) 5.7 µm	1	3.42	3.38
	2	3.66	3.64
	3	3.41	3.40
	4	3.41	4.39
	5	4.30	4.21
	6	2.87	2.83
	7	3.73	3.70
	8	4.47	4.44
	9	4.11	4.08
	10	4.13	4.11

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<u>Table</u> (continued)

Bond Wavelength	Sample	Determined fat concentration (%) Homogenized	Determined fat concentration (%) No homogenization
(C-H) 3.5 μm	1	3.44	3.48
	2	3.67	3.73
	3	3.41	3.47
	4	3.43	3.48
	5	4.40	4.42
	6	2.89	2.96
	7	3.73	3.84
	8	4.45	4.53
	9	4.11	4.20
	10	4.13	4.22

The results show that, when the determination is based on the absorption peak of the single C-O bond, the values obtained for the fat concentration within the framework of the reading precision of the apparatus are the same regardless of whether or not the sample has been homogenized before the absorption measurement. In practice the results shows that the homogenization of a sample of raw milk is unnecessary in the method according to the invention. For the sake of comparison it can be noted that also in the determination based on the absorption peak of the C-C bond the fat concentration values obtained from a homogenized sample and from an unhomogenized sample are very close to each other, although the correspondence is not as good as in the determination according to the invention, whereas in determinations based on the C=O absorption peak, and especially on the C-H absorption peak, the values obtained with a homogenized sample and an unhomogenized sample deviate from each other to such an extent that homogenization of the sample is indispensable in practical determinations.

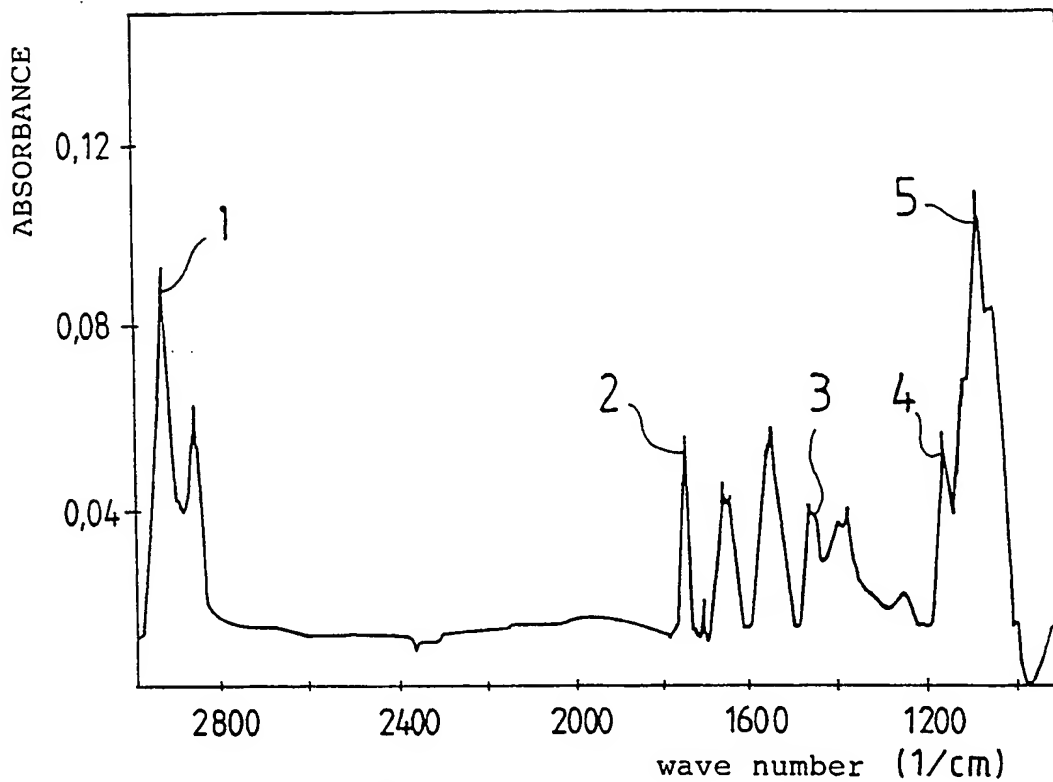It is clear for an expert in the art that the different embodi-

ments of the invention are not limited to that presented above
as an example; they may vary within the scope of the accompany-
ing patent claims. It should be noted in particular that the
coefficients of the calculation formula presented are appa-
ratus-specific values which change when a shift is made to the
use of some other spectrometer. It should additionally be noted
that, although the discussion above is mainly about the use of
the invention in the determination of the fat concentration in
milk or in milk products, emulsions containing other fats, such
as vegetable fats, can also be measured in accordance with the
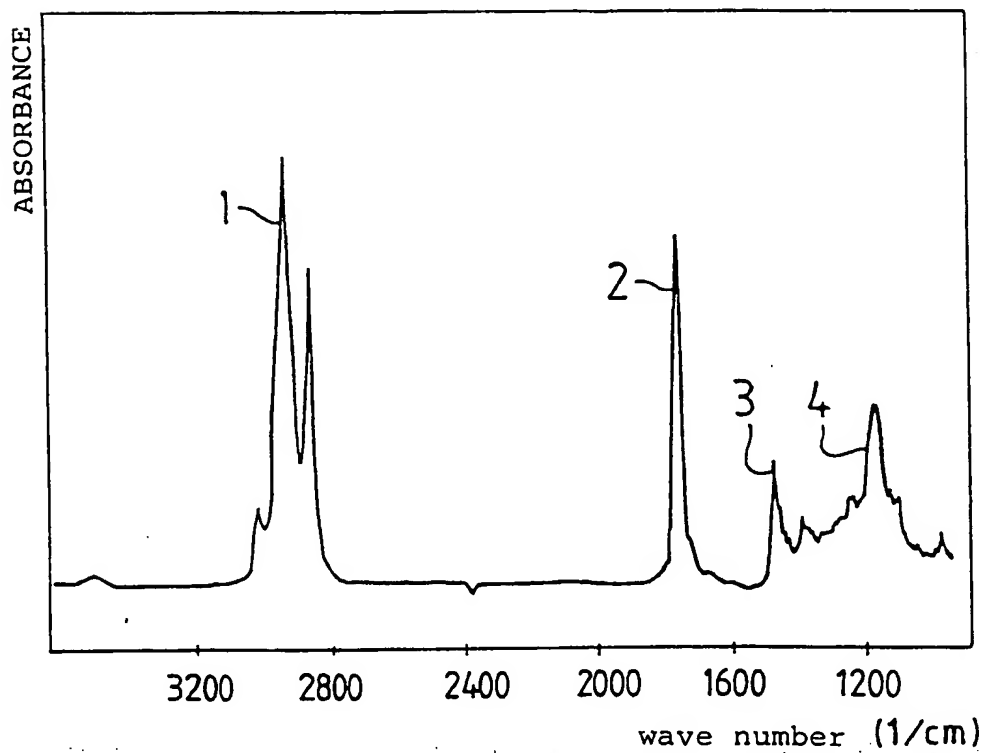invention.

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Claims

1.   A method for quantitative determination of fat from an
emulsion containing fat particles, in which method the infrared
absorption of an emulsion sample is measured and the fat is
determined on the basis of a specific absorption peak in the
absorption spectrum, by eliminating from it a any error effect
possibly caused by other constituents, characterized in that
the determination is carried out from the absorption peak, at a
wave number of approx. 1160-1190 (1/cm), of single carbon-
oxygen bonds (C-O) of the ester groups of the fat molecules.

2.   A method according to Claim 1, characterized in that the
reference used is the absorption measured at a wave number of
approx. 1200 or at a wave number of approx. 1300-1350.

3.   A method according to Claim 2, characterized in that a
continuous infrared absorption spectrum is measured at least
within a wave number range of approx. 1160-1350.

4.   A method according to any of the above claims, charac-
terized in that the emulsion is milk or a milk product.

5.   A method according to Claim 4, characterized in that the
emulsion is full milk having a fat concentration of approx.
3.0-5.5 %.

6.   A method according to Claim 5, characterized in that the
infrared absorption spectrum is measured from unhomogenized
milk in which the fat particle size varies mainly within the
range 0.5-5 μm.

7.   A method according to any of Claims 4-6, characterized
in that the absorption peak, at a wave number of approx. 1050,
of the lactose present in the milk is measured, and on the
basis of it the effect of lactose on the said absorption peak
of the carbon-oxygen bond is eliminated.

8.   A method according to Claim 7, **characterized** in that a continuous infrared absorption spectrum is measured within a wave number range of approx. 1050-1350.

Fig.1

Fig.2

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI 92/00100

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 21/35; 33/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category*	Citation of Document,[11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	US, A, 4447725 (DELMAR A. BRIGGS ET AL) 8 May 1984, see abstract	1-8
A	EP, A1, 0122749 (SHIELDS INSTRUMENTS LIMITED) 24 October 1984, see abstract	1-8
A	US, A, 4310763 (JOHN SHIELDS) 12 January 1982, see abstract	1-8
A	US, A, 4247773 (STEN A. NEXO ET AL) 27 January 1981, see abstract	1-8
A	US, A, 4076983 (JOSEPH HOPKINS ET AL) 28 February 1978, see abstract	1-8

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15th June 1992	1992 -07- 0 1

International Searching Authority	Signature of Authorized Officer
SWEDISH PATENT OFFICE	KARIN SÄFSTEN

Form PCT/ISA/210 (second sheet) (January 1985)

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on   29/05/92
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